

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings of claims in the application:

**Listing of Claims:**

1. (Previously Presented) A microfluidic device comprising:  
a plurality of first flow channels; and  
a plurality of second flow channels, each such second flow channel intersecting multiple of the first flow channels to define intersecting volumes and a plurality of looped flow channels that each include segments of the flow channels between the intersecting volumes to define a closed loop;  
a plurality of control valves, each such control valve having a control channel and a deformable segment disposed to restrict flow through a respective one of the first and second flow channels in response to an actuation force applied to the control channel to deflect the deformable segment; and  
a pump operatively disposed to regulate flow through one of said looped flow channels to regulate flow by the recirculating pump.
2. (Previously Presented) The microfluidic device of Claim 1, wherein the recirculating pump comprises multiple control channels formed within an elastomeric layer and separated from the looped flow channel by an elastomeric segment deflectable into the looped flow channel in response to an actuation force.
3. (Previously Presented) The microfluidic device of Claim 1, wherein actuation of the control valves forms a plurality of holding valves, each such holding valve being operatively disposed to form a holding space encapsulating one of the intersecting volumes.
4. (Original) The microfluidic device of Claim 1 further comprising a solution inlet for each of said first flow channels in fluid communication therewith for introduction of a first solution.

5. (Original) The microfluidic device of Claim 4 further comprising a second solution inlet for each of said second flow channels in fluid communication therewith for introduction of a second solution.

6. (Previously Presented) The microfluidic device of Claim 1, wherein the flow channels are located on an interface between a solid substrate layer and an elastomeric layer such that one side of each flow channel is formed by said solid substrate surface.

7. (Previously Presented) The microfluidic device of Claim 1, wherein:  
the flow channels are located within an elastomeric layer; and  
each of the intersecting volumes comprises a via in fluid communication with a solid substrate surface to form a well for collecting fluid.

8. (Previously Presented) The microfluidic device of Claim 1 further comprising a plurality of first flow channel pumps, each such first flow channel pump being operatively disposed to regulate solution flow through a respective one of the first flow channels.

9. (Previously Presented) The microfluidic device of Claim 8 further comprising a plurality of second flow channel pumps, each such second flow channel pump being operatively disposed to regulate solution flow through a respective one of the second flow channels.

10. (Previously Presented) The microfluidic device of Claim 9, wherein each such flow channel pump comprises multiple control channels formed within an elastomeric layer and separated from the respective flow channel by an elastomeric segment deflectable into the respective flow channel in response to an actuation force.

11. (Previously Presented) The microfluidic device of Claim 1 further comprising a first solution outlet channel in fluid communication with each of said first flow channels to receive solution flowing from each of said first flow channels.

12. (Previously Presented) The microfluidic device of Claim 1 further comprising a second solution outlet channel in fluid communication with each of said second flow channels to receive solution from each of said second flow channels.

13. (Previously Presented) The microfluidic device of Claim 1, further comprising a solid support surface having a ligand that is capable of binding to a specific antiligand at each of the intersecting volumes.

14. (Withdrawn) A method of conducting a binding assay with a microfluidic device having a plurality of first flow channels and a plurality of second flow channels, each such second flow channel intersecting multiple of the first flow channels to define intersecting volumes and a plurality of looped flow channels that each include segments of the flow channels between the intersecting volumes to define a closed loop, a plurality of control valves, each such control valve having a control channel and a deformable segment disposed to restrict flow through a respective one of the first and second flow channels in response to an actuation force applied to the control channel to deflect the deformable segment, and a recirculating pump operatively disposed to regulate flow through one of the looped flow channels to regulate flow by the recirculating pump, the method comprising:

applying an actuating force to each control channel of a first plurality of the control valves to restrict solution flow through each of the second flow channels;

introducing a reagent comprising a ligand into at least one of the first flow channels under conditions sufficient to attach the ligand covalently to a solid substrate surface;

removing the actuation force to the each control channel of the plurality of control valves and applying an actuation force to each control channel of a second plurality of the control valves such that solution flow through the each control channel of the second plurality of control valves is restricted; and

performing a binding assay by introducing a sample solution into the second flow channels under conditions sufficient to specifically bind an antiligand that may be present in the sample solution to the ligand that is covalently attached to the solid substrate surface.

15. (Withdrawn) The method of Claim 14 further comprising removing any ligand that is not attached to the solid substrate surface from the each control channel of the second plurality of control valves prior to introducing the sample solution into the second flow channels.

16. (Withdrawn) The method of Claim 14, wherein:  
performing the binding assay comprises applying an actuating force to the control valves to form a plurality of looped flow channels; and  
circulating the sample solution within each of the looped flow channels the recirculating pumps.

17. (Withdrawn) The method of Claim 14, wherein performing the binding assay comprises applying an actuating force to the control valves after introducing the sample solution into the second flow channels such that a plurality of holding spaces are formed to encapsulate each of the intersecting volumes, thereby allowing a prolonged contact between the sample solution and the ligand that is attached to the solid substrate surface the intersecting volumes.

18. (Withdrawn) The method of Claim 14, wherein:  
the flow channels are located within an elastomeric layer; and  
each of the intersecting volumes comprises a via in fluid communication with the solid substrate surface to form a well for collecting fluid.

19. (Withdrawn) The method of Claim 14, wherein: the first flow channels are in communication with a pump; and  
the reagent is transported through the first flow channels under action of the pump.

20. (Withdrawn) The method of Claim 19, wherein the pump comprises multiple control channels formed within an elastomeric layer and separated from the first flow

channels by elastomeric segments deflectable into the first flow channels in response to an actuation force, whereby the reagent is transported along the first flow channels.

21. (Withdrawn) The method of Claim 14, wherein the second flow channels are in communication with a pump; and

the sample solution is transported through the second flow channels under action of the pump.

22. (Withdrawn) The method of Claim 21, wherein the pump comprises multiple control channels formed within an elastomeric layer and separated from the second flow channels by elastomeric segments deflectable into the second flow channels in response to an actuation force, whereby the sample solution is transported along the second flow channels.

23. (Withdrawn) The method of Claim 14, wherein performing the binding assay comprises removing an elastomeric layer from the solid substrate surface and determining ligand/antiligand binding at each of the intersecting volumes with a detector.

24. (Withdrawn) The method of Claim 23, wherein the detector detects an optical signal within the intersecting volumes.

25. (Withdrawn) The method of Claim 24, wherein the detector detects a fluorescence emission, fluorescence polarization or fluorescence resonance energy transfer.

26. (Withdrawn) The method of Claim 24, wherein the detector is an optical microscope, a confocal microscope or a laser scanning confocal microscope.

27. (Withdrawn) The method of Claim 23, wherein the detector is a non-optical sensor selected from the group consisting of a radioactivity sensor and an electrical potential difference sensor.

28. (Withdrawn) The method of Claim 14, wherein performing the binding assay comprises detecting binding between a substrate and a cell receptor; a substrate and an

enzyme; an antibody and an antigen; a nucleic acid and a nucleic acid binding protein; a protein and a protein; a small molecule and a protein; a small molecule and an oligonucleotide; and a protein affinity tag and a metal ion.

29. (Withdrawn) The method of Claim 14, wherein the assay is an assay for detecting a toxic effect on cells or a cell death assay, or a cell proliferation assay.

30. (Withdrawn) The method of Claim 14, wherein the assay is an oligonucleotide binding assay or a peptide binding assay.

31. (Withdrawn) The method of Claim 14, wherein the assay is an antimicrobial assay.

32. (Withdrawn) A method for producing a microfluidic device comprising:  
producing a control layer, a flow layer, and a via layer from an elastomeric polymer, wherein each of the control layer and the flow layer comprises grooves on respective surfaces for forming control channels and flow channels; and

attaching the control layer to the flow layer such that the grooves in the control layer are attached to a top surface of the flow layer to form a plurality of control channels and attaching a bottom surface of the flow layer to the via layer to form a plurality of first flow channels and a plurality of second flow channels, wherein each first flow channels intersects multiple of the second flow channels to form a plurality of channel intersections, and wherein a via in the via layer is positioned at each channel intersection.

33. (Withdrawn) The method of Claim 32, wherein producing the via layer comprises etching the via layer to produce a plurality of vias.

34. (Withdrawn) The method of Claim 32, further comprising attaching the elastomeric polymer to a solid substrate that comprises a ligand bound to its surface or comprises a functional group capable of attaching a ligand.